DNA Photoswitching by Azobenzene

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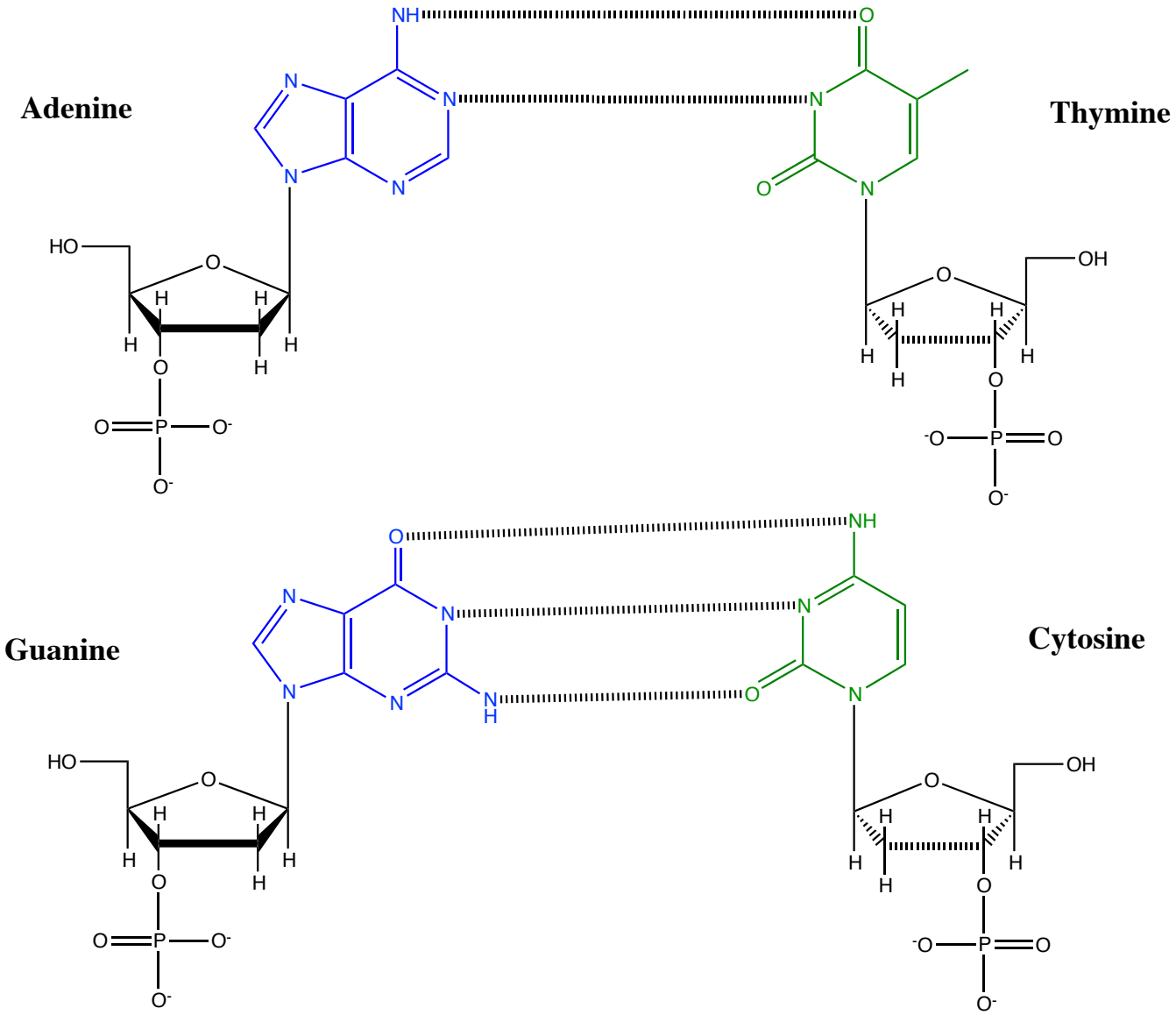
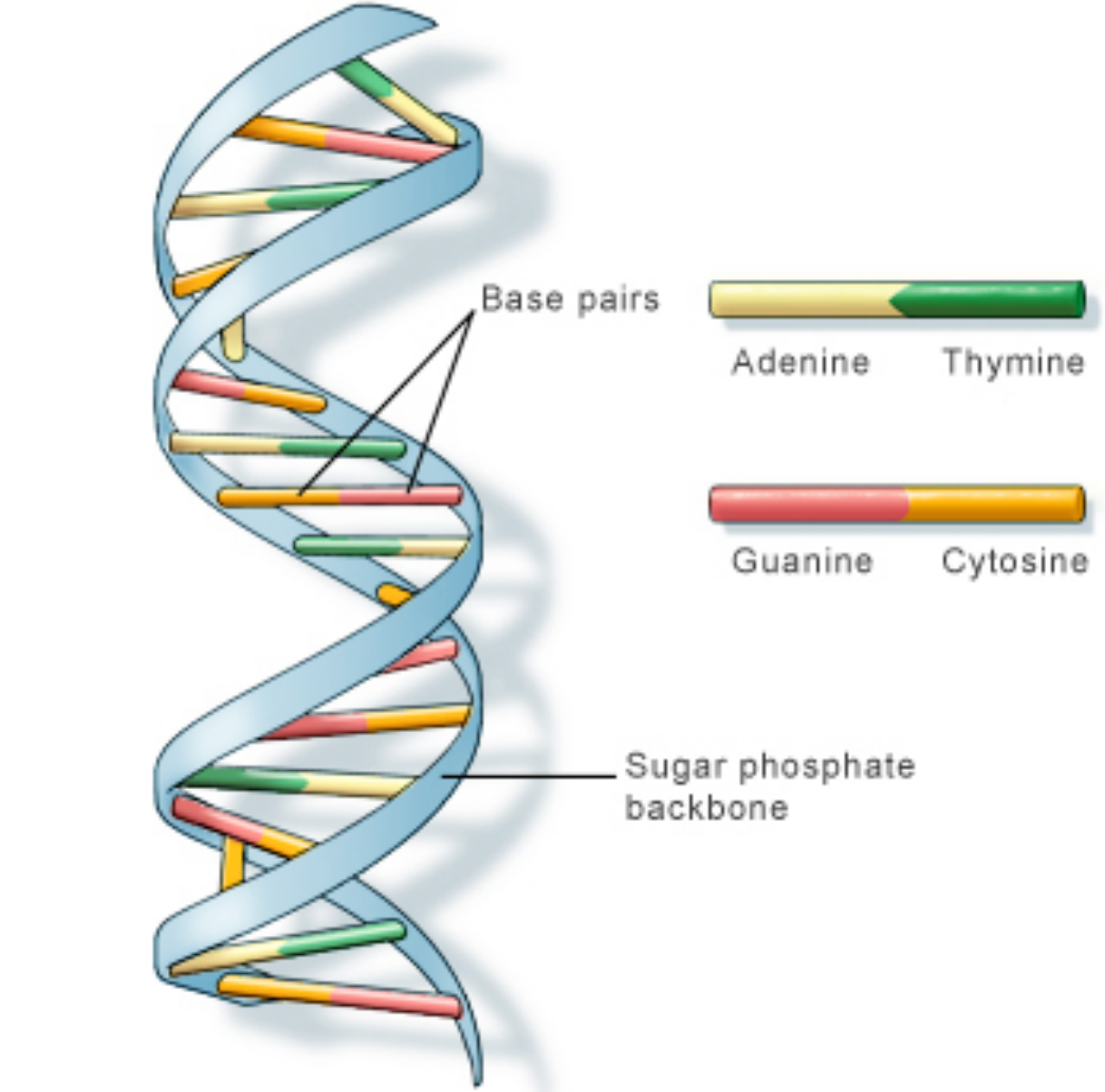
DNA

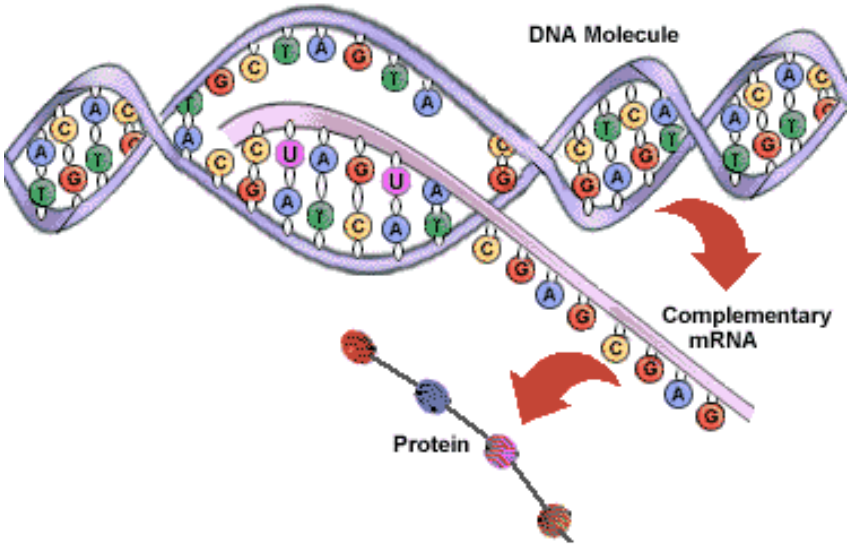


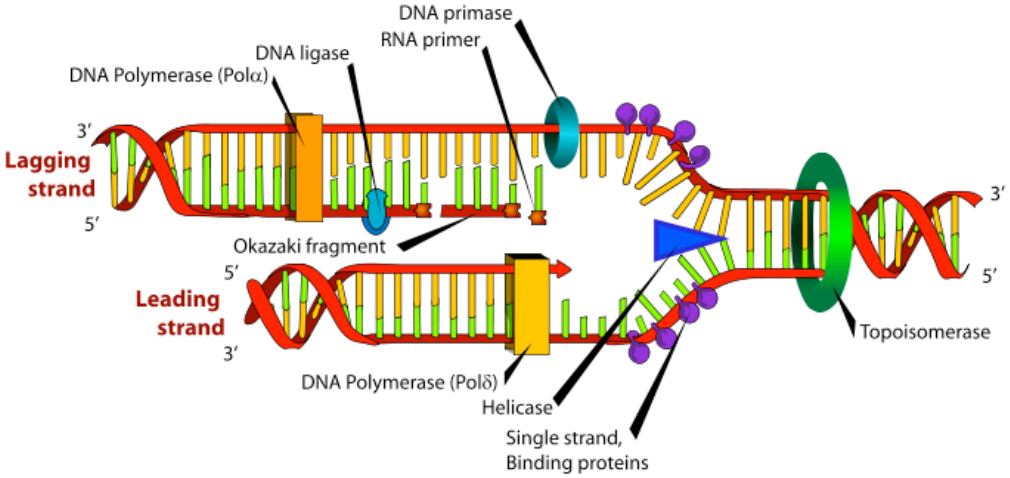


U.S. National Library of Medicine

- Transcription of genes and replication of DNA

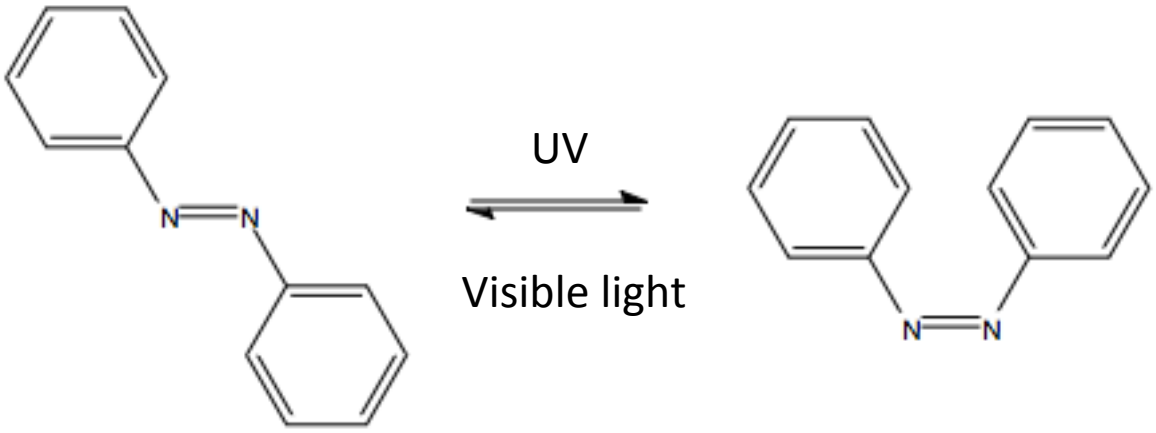
- Separation of DNA strands: must expose strands to pair new bases correctly





Azobenzene and photoswitching

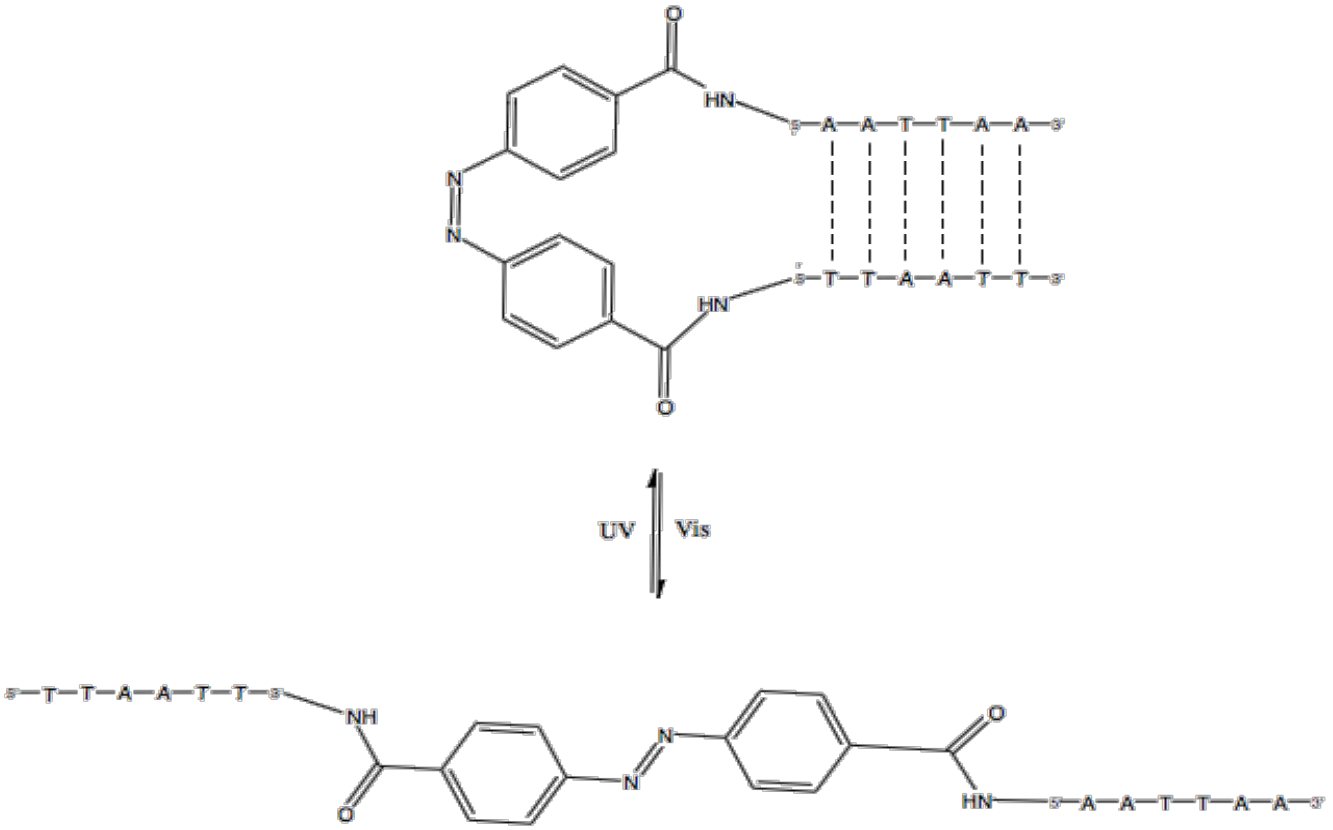
- 2 benzene rings, connected by azo group
- Under UV light switches from *trans* to *cis* form



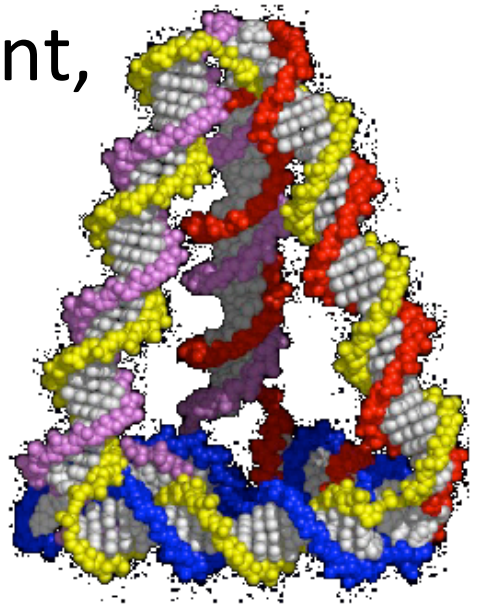
- Switch takes on the order of picoseconds

Why combine DNA and azobenzene?

- Maybe azobenzene can replace DNA helicase in synthetic systems:



- Could form part of an artificial ribosome project, and let us open and close DNA whenever we want, without enzymes.

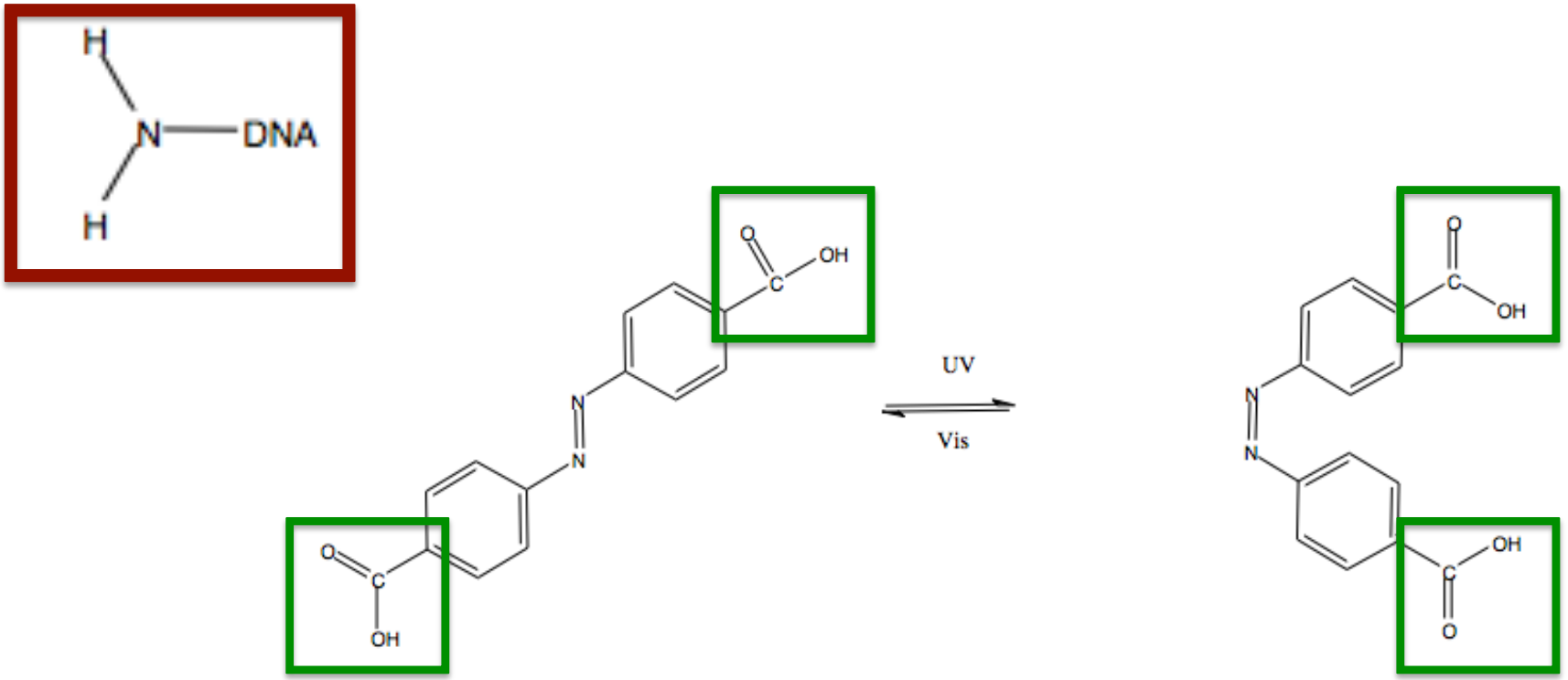


- Also useful in DNA origami.

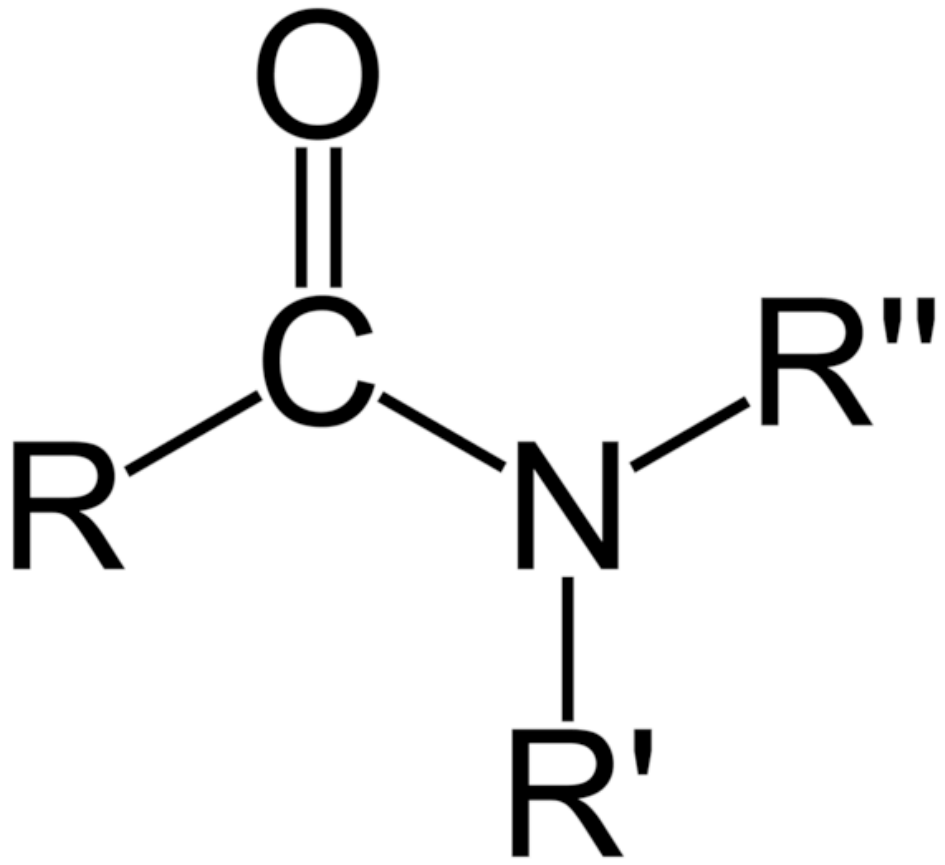
- Or any system where tight control of DNA's shape is needed, especially if it needs to change.

How can we combine them?

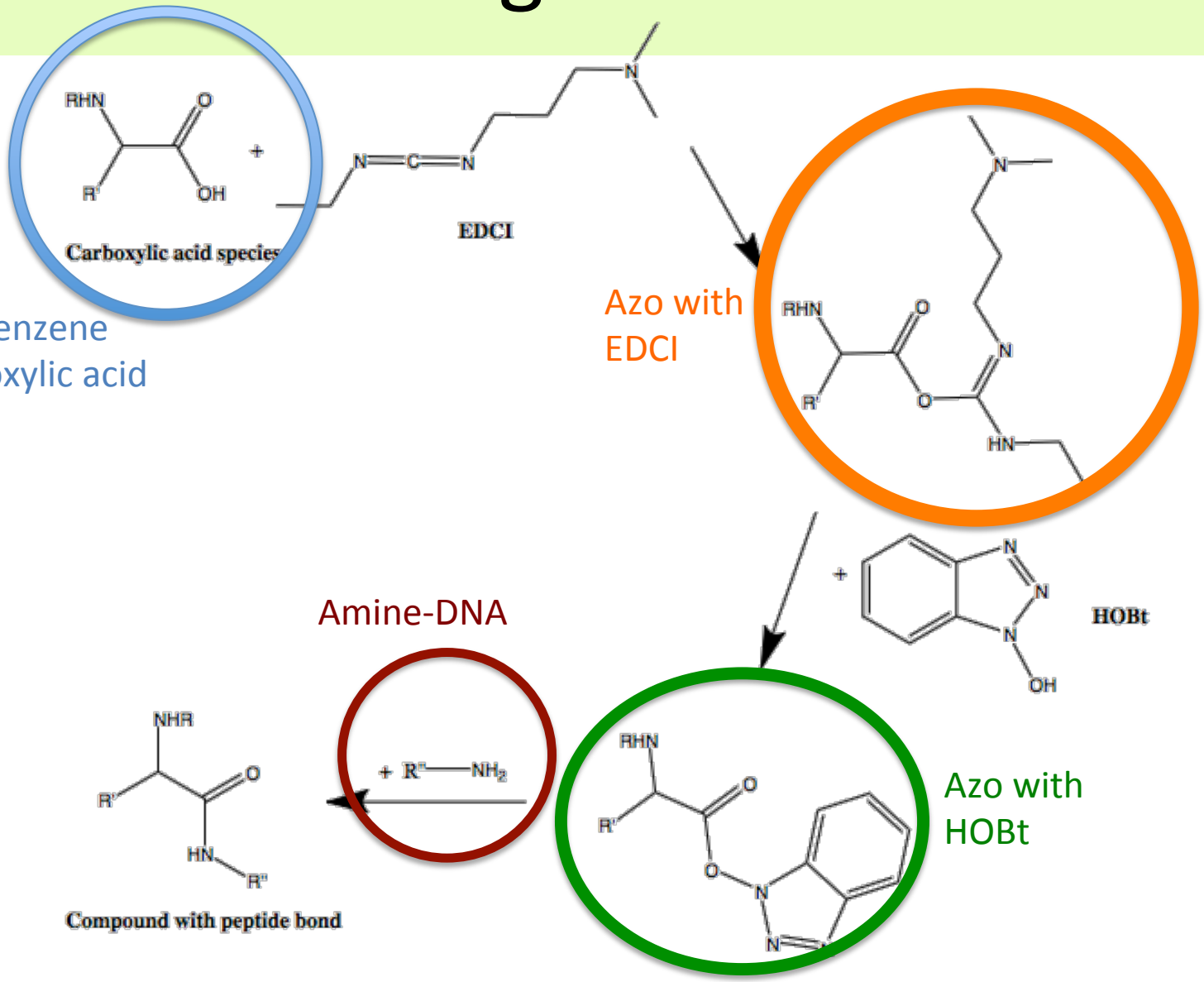
- DNA strands and azobenzene both modified: amine on DNA, carboxylic acids on azobenzene



- The modifications allow us to form amide bonds (called peptide bonds in proteins):



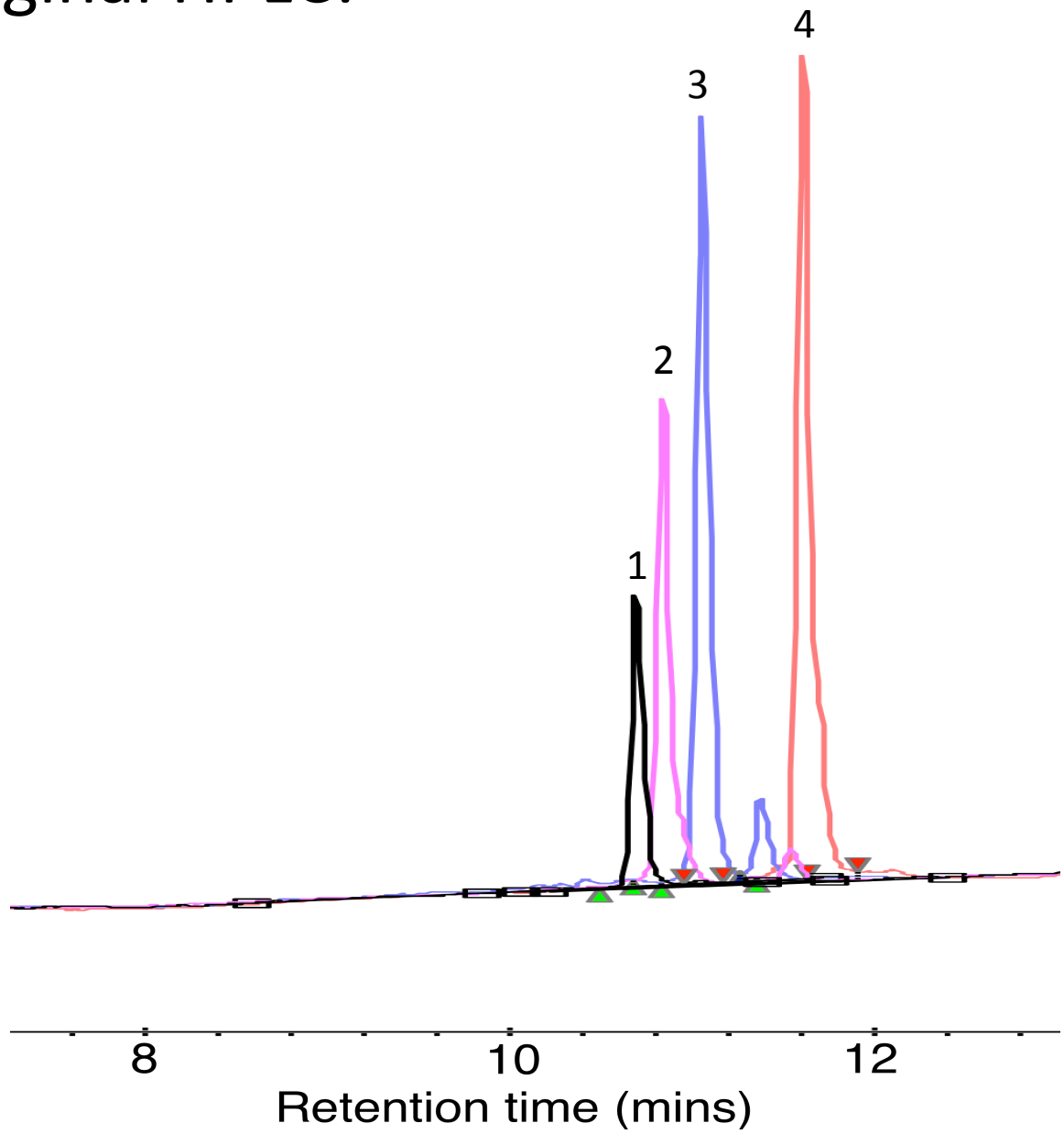
Forming Amide bonds



Identifying products

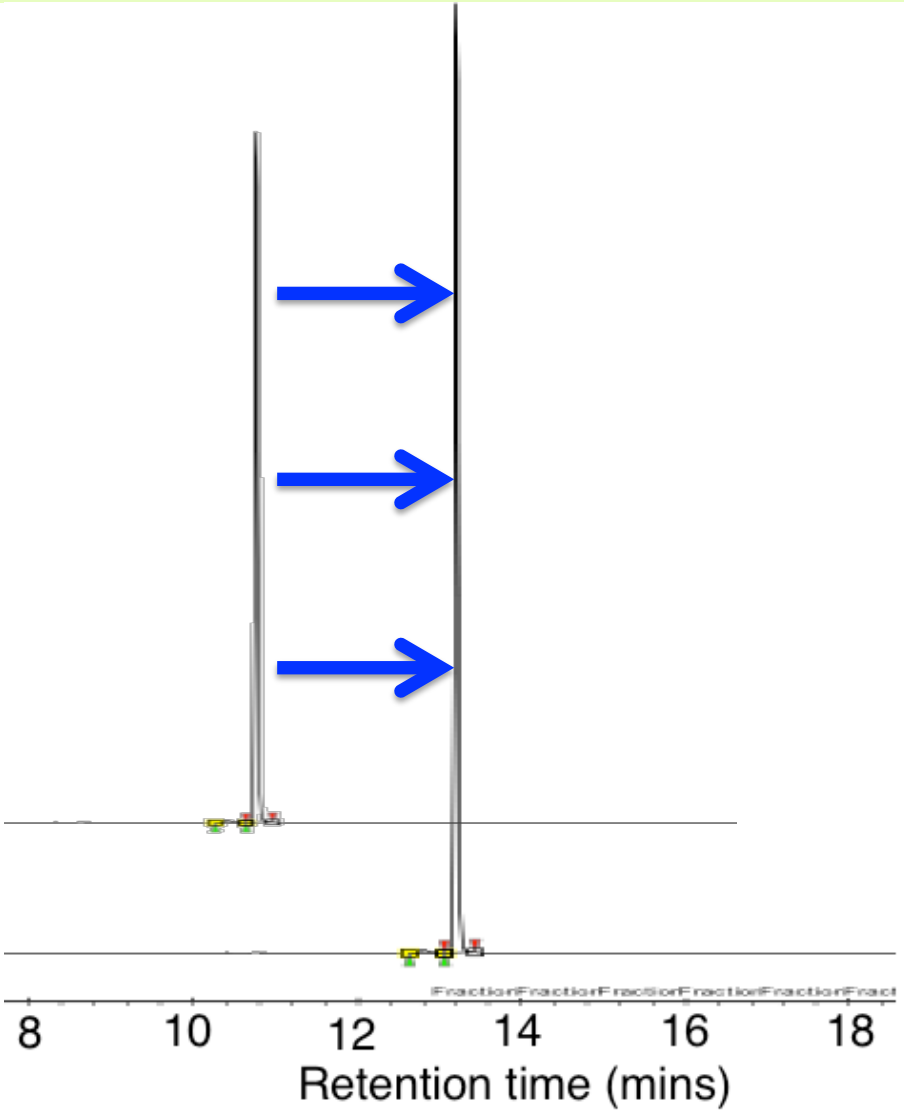
- High Performance Liquid Chromatography
  - Sample pushed through column of semi-porous material.
  - According to size and chemistry, molecules detected at end of column at specific times…
  - We look for new peaks

- Original HPLC:



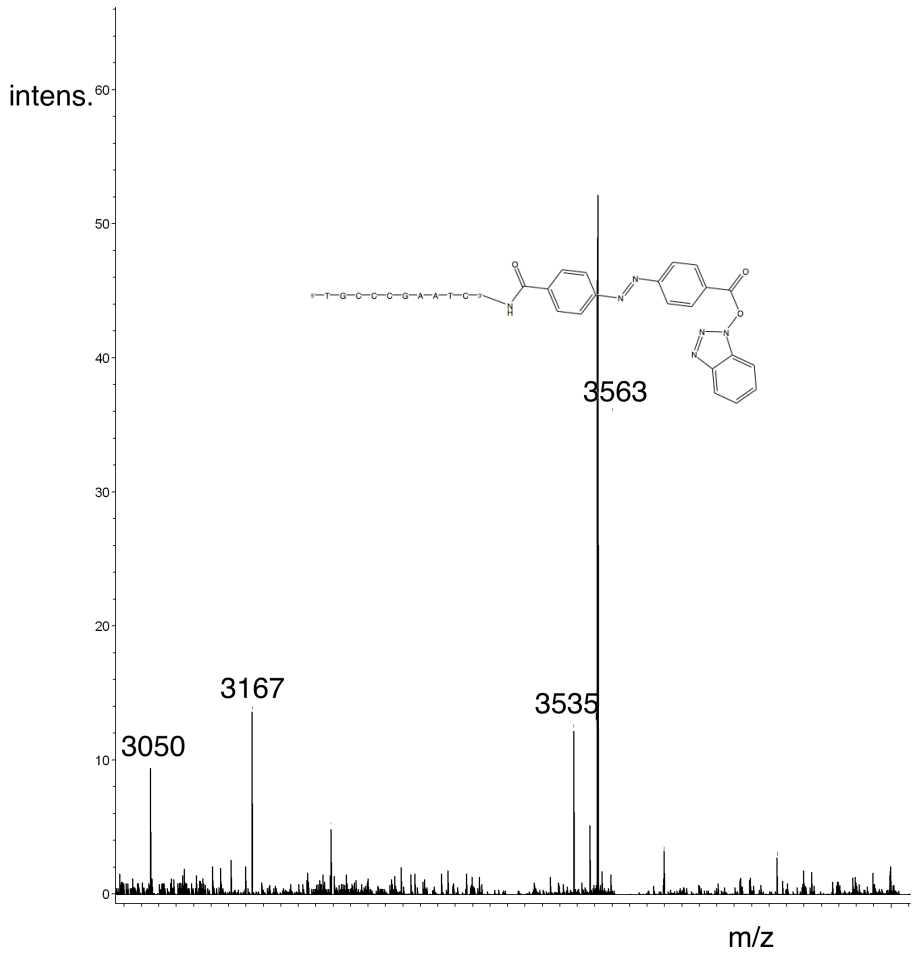
1st attempt

- Reacted DNA strand 1 with azobenzene.
- Used HPLC to see if product made.
- Shift in peak location => success?



Was the product right?

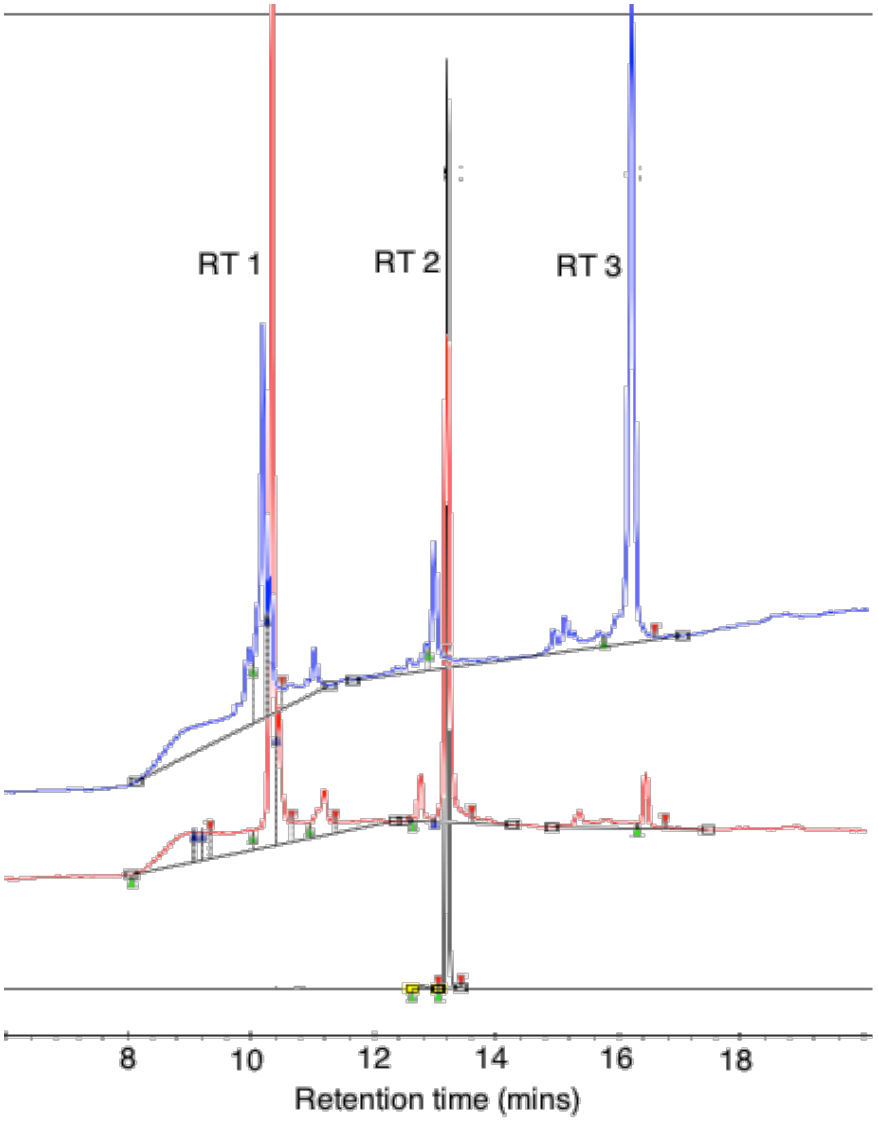
- Used Mass Spectrometry to estimate the mass of compounds present after the reaction.
- 3563 is correct! (the mass of DNA1 + Azo + HOBt)



Adding complementary DNA

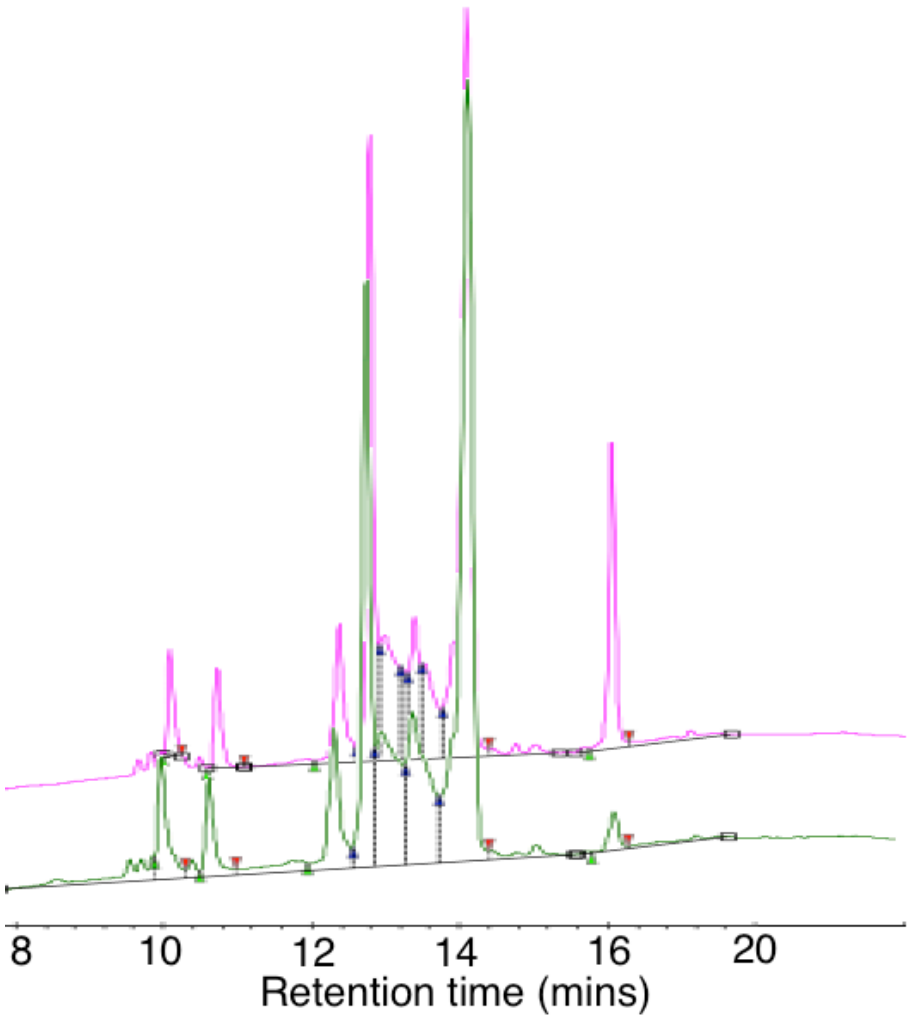
- Tried adding DNA strand 2 with more coupling reagents... Peak for DNA1-azo (middle), DNA 2 (left), nothing else significant.

- Added more coupling reagents... 3rd peak seen (right)

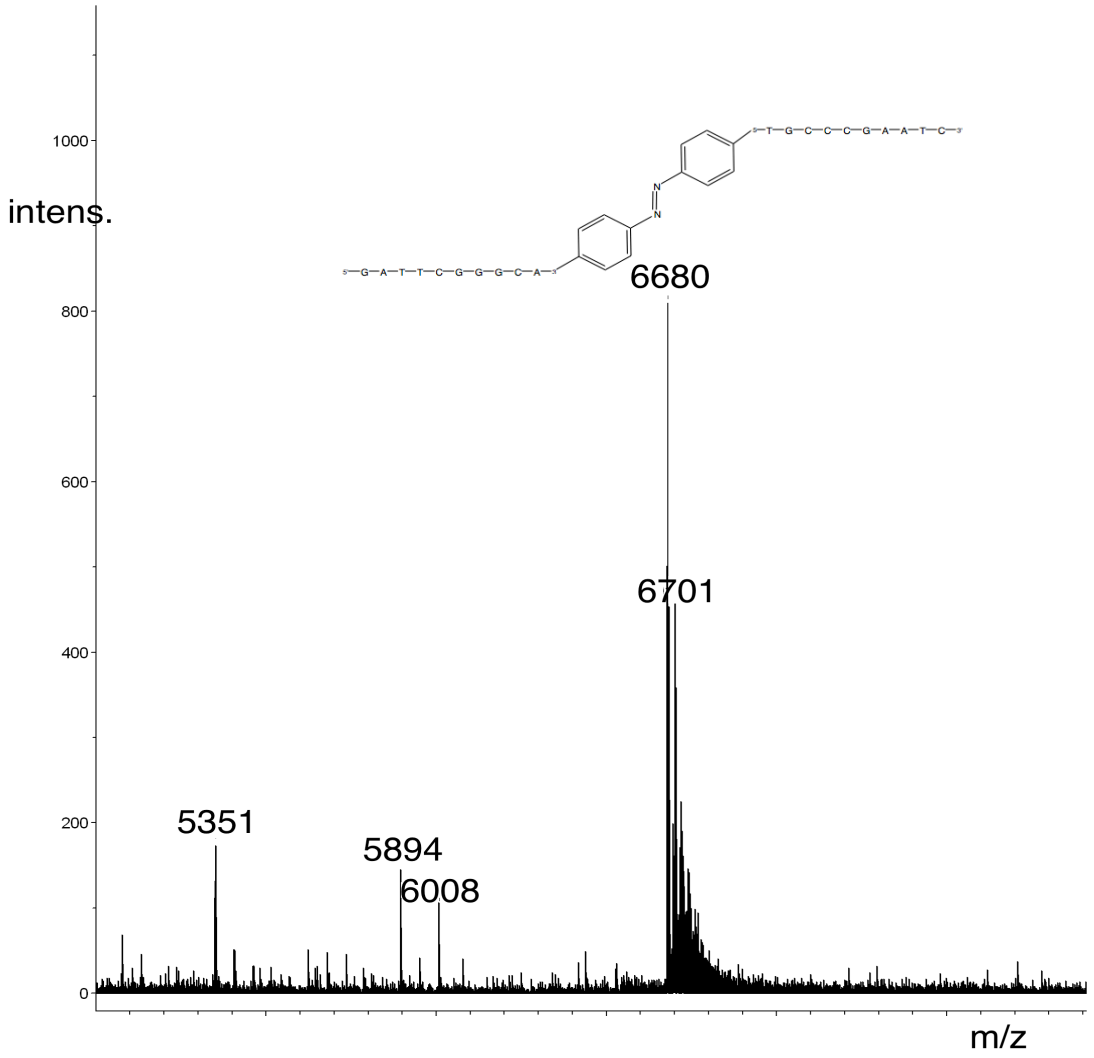


Another method

- DNA strands 1 and 2 allowed to hybridise first
  - Should make it easier to bond with both DNA strands at once
  - Should reduce chance of other molecules forming
- Tried reaction with UV light and without UV light



New peak tested



- Mass is exactly as expected for DNA-Azo-DNA compound

Conclusions

- Using modified DNA and azobenzene, with the coupling reagents HOBt and EDCI, we can form amide bonds between DNA strands.
- These strands can be complementary, but other implications of this are yet to be tested.

Future work

- Repeat with different sizes of DNA strand.
- Introduce other techniques for characterisation (*e.g.* gel electrophoresis)
- Test products for azobenzene photoswitching:
  - Do we see DNA duplex being pulled apart?
  - How long a section of DNA will the azobenzene separate in this way before it is overwhelmed by the strength of hydrogen bonds?

Thanks

- Phillip Milnes
- Rachel O'Reilly
- Tom Wilkes
- ROR Group

(www2.warwick.ac.uk/fac/sci/chemistry/research/oreilly/oreillygroup)

- EPSRC & MOAC

Main References:

1. E. Valeur and M. Bradley, Chem. Soc. Rev, 2008, 38, 606 – 631.
2. A. Beharry and G. Woolley, Chem. Soc. Rev, 2011, 40, 4422 – 4437.